Interleukine-6, Vitamins A, E, and C Levels in Serum of Iraqi Patients with Fibromyalgia Syndrome (FMS)

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ABSTRACT

The main objective of this study is to determine the concentrations of Interleukine-6, Vitamins A, E, and C in patients with FMS. This study included 60 patients with FMS were diagnosed according to the American college of Rheumatology (ACR) 1990 criteria. They were obtained from the people attending the Rheumatology & Rehabilitation Consultation Unit / Out Patient Clinic/ Baghdad Teaching Hospital in Medical City. Their ages range were 20-60 years and their ages range were then matched by age and sex to 30 healthy control persons with mean age 43.25 ± 3.16 years. Results revealed that there was no significant difference in the mean value of IL-6 in serum of patients with FMS, while vitamins A, and E concentrations in serum of patients with FMS were significantly decreased as compared to the level in serum of healthy controls ($p>0.05$), and the mean values of Vitamin C concentration in serum of patients with FMS were significantly increased than the level in serum of healthy control ($p< 0.05$).

Key ward: IL-6, Vitamins A, E, C, and FMS

مستويات أنترليوكين -6 وفيتامينات A، E، C في مصل مرضى عراقيين مصابين بمتلازمة الألم الليفي العضلي

الخلاصة

الدراسة تشمل ٦٠ من المرضى المصابين بمتلازمة الألم الليفي العضلي تم تشخيصهم بموجب معيار كلية الروماتيزم الأمريكية لعام ١٩٩٠ تتراوح اعمارهم بين ٢٠-٦٠ سنة، تتطابق أعمار وجنس المرضى مع ٣٠ من الأشخاص الأصحاء وبمعدل عمر يتراوح بين ٤٣،٢٥± ٣،١٦ سنة. النتائج تشير الى أنه ليس هناك أختلاف في متوسط قيم تراكيز أنترلوكين-٦ بين المرضى والأصحاء بينما متوسط قيم تراكيز فيتامينات A، E في أمصال المرضى المصابين بمتلازمة الألم الليفي العضلي تنخفض بصورة مؤثرة ($p>0.05$) مقارنة بالمستوى في الأشخاص الأصحاء، بينما متوسط قيم تراكيز فيتامين C في أمصال المرضى المصابين بمتلازمة الألم الليفي العضلي تزداد بصورة مؤثرة ($p< 0.05$) مقارنة بالمستوى في الأشخاص الأصحاء.

الكلمات الدالة: متلازمة الألم الليفي العضلي، أنترليوكين-٦، فيتامينات A، E و C

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INTRODUCTION

Fibromyalgia syndrome (FMS) is a common, chronic widespread pain syndrome usually associated with other somatic and psychologic symptoms including fatigue, sleep disturbances, cognitive difficulties (memory problems, diminished mental clarity, and concentration difficulties) and cite conditions such as anxiety, depression and Posttraumatic Stress Disorder and other core symptoms include debilitating fatigue, sleep disturbance, and joint stiffness [1]. Also FMS is a chronic musculoskeletal syndrome; almost invariably, symptoms persist at 5- and 10-year follow-ups. The degree of functional impairment is similar to that seen in patients with moderate to severe rheumatoid arthritis [2,3]. Fibromyalgia is characterized by chronic widespread pain and also allodynia, a heightened and painful response to pressure [4]. Other core symptoms include joint stiffness. Some patients [5] may also report difficulty with swallowing [6], bowel and bladder abnormalities [7]. FMS affects 2-4 % of general population, and affects more females than males, with a ratio of 9:1 by American college of Rheumatology criteria (ACR) [8].

IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response, during infection and after trauma, especially burns or other tissue damage leading to inflammation. In terms of host response to a foreign pathogen during infection [9]. IL-6 is also a "myokine," a cytokine produced from muscle, and is elevated in response to muscle contraction [10]. It is significantly elevated with exercise, and precedes the appearance of other cytokines in the circulation. During exercise, it is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery. Additionally, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on tumor necrosis factor-alpha (TNF-alpha) and IL-1, and activation of interleukin-1 receptor antagonist (IL-1ra) and IL-10 [11].

Vitamin A (Retinol) is serving a number of critical physiological roles, as evidenced by myriad of disorders that accompany deficiency or excess states. Also it has a structure depicted to the right retinol is the immediate precursor to two important active metabolites: retinal which plays a critical role in vision, and retinoic acid, which serves as an intracellular messenger that affect transcription of a number of genes. Vitamin A deficiency usually results from malnutrition, but can also be due to abnormalities in intestinal absorption of retinol or carotenoids [12].

Vitamin E is fat soluble substance present in cellular membrane. It is a powerful antioxidant, protect cells from oxidation and neutralize unstable free radical which can cause damage. This is done by vitamin E giving up one of the electrons to the electron deficient free radical making it more stable. The major function of vitamin E in the body is an antioxidant and acts primarily by scavenging active oxygen free radicals, which otherwise attack substrates such as

lipids, proteins, sugar and DNA to initiate damaging chain reaction. It may also stimulate immune response, inhibit cancer initiation, and inhibit the conversion of nitrite to nitroamines, which are strong promoters of tumor formation. Also vitamin E protects the other antioxidant from being oxidized. The capability is great in helping to prevent the degenerative diseases; including heart disease, strokes, arthritis, diabetes, and cancer [13].

Vitamin C (Ascorbic acid) is an important water soluble antioxidant found in biological system. It is the first line antioxidant defense in plasma. Ascorbic acid has many functions including prevention of scurvy, acceleration of hydroxylation reaction in the synthesis of collagen in connective tissue, carnitine and nor epinephrine, amidtion of peptide hormones, regeneration of vitamin E, protection against photo oxidative damage, conversion of cholesterol to bile acids and enhance iron bioavailability. Vitamin C is a great antioxidant protects the body against pollutants. Vitamin C is a biological reducing agent; it also linked to its prevention of degenerative diseases [14].

Patients & Methods

The prospective study comprised 60 Iraqi patients of FMS (50 female, 10 male) fulfilling the American College of Rheumatology (ACR) 1990 criteria for the diagnosis of FMS, were obtained from the people attending the Rheumatology & Rehabilitation Consultation Unit / Out Patient Clinic/ Baghdad Teaching Hospital in Medical City, their ages range were then matched by age and sex to 30 healthy control persons (22 female, 8 male). The laboratory tests were done in Teaching Laboratories of the Medical City and the Department of Physiological Chemistry / College of Medicine University of Baghdad. All subjects non smokers and were evaluated by BMI (weight in kilograms divided by the square of height in meters). Subjects were excluded if they showed medication or had any evidence of metabolic disease other than obesity. Blood samples were taken from individuals in both groups for estimating IL-6, vitamins A, E, and C concentrations. Laboratory investigation which include: Hemoglobin (Hb), Erythrocyte sedimentation rate (ESR) was done in Laboratory Teaching center of Baghdad Hospital. The IL-6 Kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for IL-6 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-6 concentrations, control specimens and unknowns are pipetted into these wells. During the first incubation, the IL-6 antigen and a biotinylated monoclonal antibody specific for IL-6 are simultaneously incubated. After washing, the enzyme (streptavidin - peroxydase) is added. After incubation and washing to remove all the unbound enzyme, a substrate solution which is acting on the bound enzyme is added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL-6 present in the samples; the IL-6 Kit was from HUMAN-Germany [15].

Vitamins A, E, and C levels were measured using high performance liquid Chromatography (HPLC). Fat soluble vitamins A and E were separated on NH_2 column isocratically after obtaining optimum conditions (temperature, flow rate, and eluent composition), eluent used for vitamins A and E was Tetrahydrofuran

(THF), methanol (MeOH), water (H_2O), while water soluble vitamin C was separated using reversed phase C-18 column, eluent used for vitamin C was acetic acid (HOAC) and acetonitrile.

Vitamin A and E conditions: Flow rate: 1ml/min. Temperature: Room temperature, Detection: UV 285 nm., Mode: Isocratic, Column: NH_2

Vitamin C conditions: Flow rate: 1ml/min., Temperature: Room temperature, Detection: UV 285 nm., Mode: Isocratic, Column: C-18.

Chromatograms were recorded and concentration of vitamins was determined from the area under the curve using standard [16].

Descriptive statistics for all data were expressed as mean±SD, and the percent of abnormal value in any test was calculated as above or below the mean±SD of the normal values for the matched control group, compared using independent sample (t) test $P < 0.005$, and considered statistically significant [17]

Results

The characteristics of 60 FMS patients and 30 controls are shown in Table (1). There was no significant difference in the mean value of IL-6 in serum of patients with FMS, while vitamins A, and E concentrations were significantly lower ($P < 0.01$), while the mean value of Vitamins C concentration were significantly higher as compared to the levels in serum of health control group ($P < 0.05$) as shown in Table (2) and Figure (1, 2).

Discussion

There was no significant difference in the concentration of interleukin-6 in patients with Fibromyalgia Syndrome (FMS) and control groups and this is in agreement with the study done by Gur A, Karakoc M, Nas K, et al. [18] that illustrated no difference between patients with FMS and control group in serum level of IL-1 and IL-6. The immune system is thought to play a role in the pathogenesis of fibromyalgia through its interactions with the (hypothalamus-pituitary axis) HPA and (central nerve system) SNS, but the precise mechanisms continue to be elucidated. Numerous studies have examined the overall function of the immune system in patients with fibromyalgia. Also our results are in agreement with numerous studies having examined the overall function of the immune system in patients with fibromyalgia. A subset of patients with fibromyalgia exhibit low-level autoantibodies (antinuclear antibody) or immunoglobulins (Ig; rheumatoid factor) and may have evidence of low-level systemic inflammation [19]. Most recent publications indicate that in FMS patients there is some abnormality with the hypothalamic-pituitary-adrenal (HPA) axis, with elevated activity of corticotropin-releasing-hormone (CRH) and substance P (SP) [20] that may not only affect the PHA axis, but other endocrine and immune processes [21]. In view of this finding and the observation that FMS symptoms commonly occur after a psychological or inflammatory stressor, FMS may be another inflammatory disorder exacerbated by stress [22]. For instance, pain perception appeared to be enhanced by the concurrent presence of stressful events [23], and repeated sound stress was shown to increase inflammatory hyperalgesia in rats [24]. Other recent publications have reported elevated levels of cytokines in the serum of FMS patients [25]. In a study, serum IL-1 and IL-6 levels were not different from controls but IL-8 was significantly elevated [26].

Nevertheless, IL-6 was elevated in the supernatants from peripheral blood mononuclear cells from FMS patients [27]. (Moreover, injection of IL-6 produced excessive heart rate responses in FMS patients [28]. Interestingly, young FMS patients with milder symptoms had significantly increased serum IL-8 levels [29].

Results obtained in this study showed that the serum levels of vitamins A and E were significantly lower in patients with FMS than in controls, while the serum level of vitamin C was significantly higher in patients with FMS than in controls. Antioxidants can help to protect the neural tissue from damage, induced by inflammatory cascade that result in free radical pathology and oxidative stress [30].

Vitamin C plays a pivotal role as a chain breaking antioxidant, and plasma lipid peroxidation is prevented only as long as vitamin C is present. Its role as antioxidant is indicated by its known free radical scavenging action. As a reducing and antioxidant agent, it directly reacts with various lipid hydroperoxides, and prevents the oxidative modification the cytosolic and membrane components of cells [31.

Vitamin A is a fat soluble vitamin and appears to protect cells against inflammatory disease, perhaps by its antioxidant properties. Supplementation of therapeutic doses of vitamin E increases the plasma level of vitamin A and beta carotene, perhaps by their synergetic interaction [32]. The available evidence suggests that vitamin A metabolism is linked to vitamin E as an antioxidant in the stability of biological membranes. In the current study, levels of vitamin A and E in serum of patients with fibromyalgia were lower than control. This may indicate the role action of vitamin A and E on radical inhibition. Results in this study agree with the study of Kamanli et al, 2004 [33] who found that the level of vitamin A and E were lower in serum of patients of Rheumatoid arthritis and agree with the study of Kokcam et al, 2002 [34] that the levels of vitamin A and E were lowtser in serum of patients of Behcet disease, and disagree with Eisinger et al, 1998 who found that there were no difference in vitamins concentration in patients and control [35].

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APPENDIX

Table (1) characteristics of FMS patients and controls.

Parameters	Healthy Control (n= 30) Mean ± SD	FMS patients (n= 60) Mean ± SD	P-value	Significant
Age (y)	43.25 ± 3.16	40.01 ± 2.10	0.41	NS
BMI (kg/m^2)	26.54 ± 0.87	27.43 ± 0.36	0.19	NS

Table (2) Mean±SD values of serum IL-6, Vitamins A, E, and C in patients with FMS and controls.

Parameters	Healthy control (n=30) Mean±SD	FMS patients (n=60) Mean±SD	P-value	Significant
IL-6 (pg/ml)	4.940±0.648	5.010±1.067	0.430	NS
Vitamin A (µg/ml)	4.452±0.165	2.864±0.144	0.001	HS
Vitamin E (µg/ml)	7.308±0.167	2.956±0.691	0.001	HS
Vitamin C (µg/ml)	8.012±0.252	8.861±0.165	0.045	S

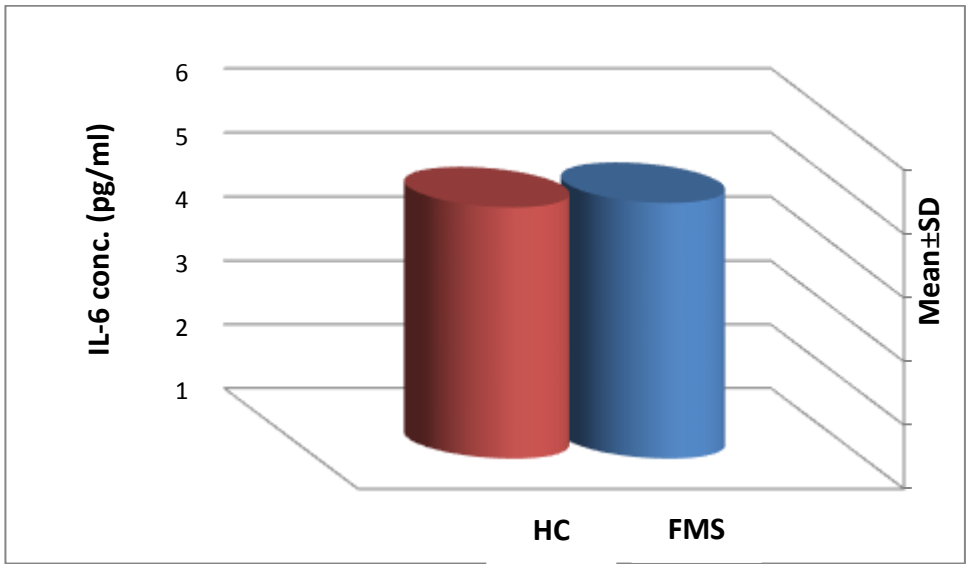

Figure (1) Interleukine-6 (IL-6) concentration in patients with fibromyalgia syndrome (FMS) and healthy control (HC).

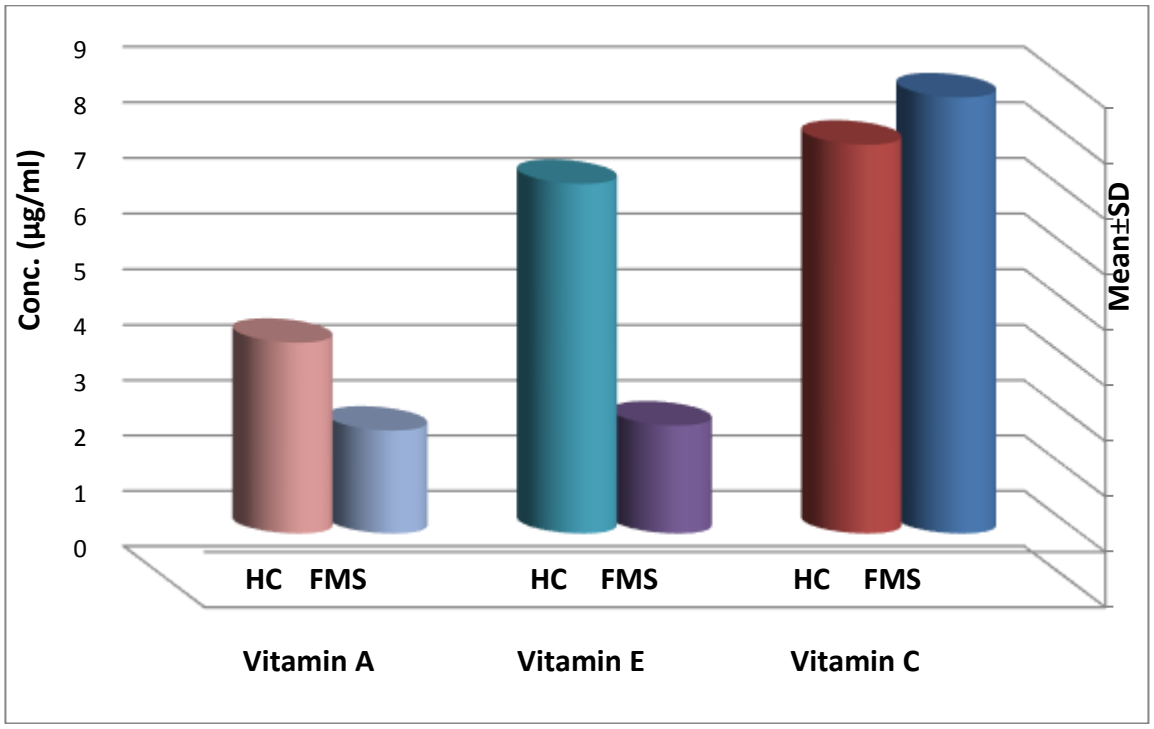

Figure (2) Vitamins C, E, and C concentration in patients with fibromyalgia syndrome (FMS) and healthy control (HC).